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BASF Aktiengesellschaft  
Product Safety  
Regulations, Toxicology and Ecology

**BASF**



**STUDY TITLE**

Report



**N-Methylpyrrolidon - Determination of the chronic effect  
on the reproduction of the water flea *Daphnia magna* STRAUS**

**DATA REQUIREMENT**

OECD 202

**AUTHOR**

Dr. Jatzek (Study Director)

**STUDY COMPLETED ON**

25 July 2001

**PERFORMING LABORATORY**

Experimental Toxicology and Ecology  
BASF Aktiengesellschaft  
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**LABORATORY PROJECT IDENTIFICATION**

Project No.: 00/0969/51/1

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## 2 QUALITY CRITERIA

This project was performed according to the following quality criteria:

- European Standard EN 45001 "General criteria for the operation of testing laboratories"
- International Standard ISO 9002 "Quality systems - Model for quality assurance in production installation and servicing"
- OECD Principles of Good Laboratory Practice (GLP).

The test facility is registered for GLP and its ecological part is accredited to Deutsche Akkreditierungsstelle Chemie GmbH (DACH) see number DAC-P-0030-97-02.

Copies of the certificates can be provided by request.

According to the International Standard ISO 31-0 the decimal sign is a comma.

### 3 SUMMARY

N-Methylpyrrolidon - Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS was investigated in a 21 day semistatic test according to OECD-Guideline for testing of chemicals, No. 202, 4. April 1984: Daphnia sp., Akute Inmobilisation Test and Reproduction Test. Part.II –Reproduction Test.

#### TEST RESULTS

|                 |   |           |
|-----------------|---|-----------|
| NOEC            | = | 12,5 mg/l |
| LOEC            | = | 25 mg/l   |
| LC <sub>0</sub> | = | 25 mg/l   |

The test substance was tested in the range of concentrations between 0,39 and 100 mg/l.  
The dilution factor was 2.

As test criteria the reproduction and mortality of the test animals are given.

#### Remarks:

With an analytical recovery rate of > 80% no transformation of the nominal EC-values into the values of the concentration control analysis occurs.

O<sub>2</sub>-concentration at day 21 in test concentration 100 mg/l was only 2,2 mg/l. This has no influence on the test results because NOEC and LOEC were in a lower concentration range.

### 4 GLP-STATEMENT OF COMPLIANCE, SIGNATURES AND RETENTION OF RECORDS

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act).

The raw data, protocol, reserve sample and the original of this report will be stored at BASF Aktiengesellschaft 67056 Ludwigshafen/Rhein (Germany) at least for the period of time specified in the GLP regulations. Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

|            |                    |                |                    |
|------------|--------------------|----------------|--------------------|
| Date:      | 26 July 2001       | 25 July 2001   | 25 July 2001       |
| Signature: | Mrs. Kary          | Dr. Slatzek    | Dr. Pappa          |
| Name       | Mrs. Kary          | Dr. Slatzek    | Dr. Pappa          |
| Function   | Project Technician | Study Director | Head of Laboratory |

**STATEMENT**  
of the Quality Assurance Unit

The Quality Assurance Unit (QAU) inspected the study and reported any inspection results to the Study Director and to Management.

The final report reflects the raw data.

| Phase of study    | Date of inspection<br>(mm-dd-yyyy) | Reported to Study Director<br>and to Management<br>(mm-dd-yyyy) |
|-------------------|------------------------------------|---|
| Study Plan:       | 03-13-2001                         | 03-13-2001  |
| Conduct of study: | 03-14-2001                         | 03-14-2001  |
| Report:           | 06-28-2001                         | 06-28-2001  |

Ludwigshafen, 25 July 2001

*Vetter*

Vetter

## 6 DESCRIPTION OF THE TEST METHOD

### Test organism

The clone of *Daphnia magna* STRAUS 1820 used was supplied by the Institut National de Recherche Chimique Appliquée, France, in 1978. From this date on this clone was cultured and bred continuously in the Laboratory of Ecology of BASF AG in Ludwigshafen.

*Daphnia magna* is geographically widely distributed and is an important part of aquatic food webs where it can be grouped between primary producers and decomposers, on the one hand, and predators, on the other. *Daphnia magna* is recommended as a test organism all over the world.

This study investigated the influence of the test substance on the reproduction and the swimming ability (mobility) of *Daphnia magna*. The test substance was suspended in water. The daphnids were exposed to various concentrations of the test substance and compared to a control.

The daphnid tests are performed under the same environmental conditions as the culture itself. Therefore a phase of adaptation for the young is not necessary.

### Definitions

Parent animals are those animals stocked at the start of the test.

Young are the progeny produced by the parent animals.

Parthenogenetic eggs are parthenogenetically produced eggs which develop without fertilization to young daphnids. Parthenogenetic eggs which fail to develop to embryos are called aborted eggs.

The LOEC (lowest observed effect concentration) for reproduction is the lowest tested concentration at which compared to an untreated control - the mean cumulative number of live young produced per parent animal that survived the test is significantly ( $\alpha \leq 0.05$ ) less than in the control. Each tested higher concentration must have at least an effect equal or stronger than the LOEC.

The NOEC (no observed effect concentration) is the tested concentration immediately below the LOEC.

The LC<sub>50</sub> is the highest concentration tested at which no difference in mortality of the parent animals was observed in relation to the control.

## 7 TEST METHODS

EEC Guideline XI/691/86, Draft 4: "Prolonged toxicity study with *Daphnia magna*: Effects on reproduction".

Deutsches Institut für Normung: Bestimmung der biologischen Wirkung von Wasserimmissionstoffen auf Kleinkrebse (Reproduktionstest mit *Daphnia magna*), DIN 38 412 (Entwurf) (1981)

OECD-Guideline for testing of chemicals, No. 202, 4 April 1984: *Daphnia* sp., Acute Immobilisation Test and Reproduction Test Part II – Reproduction Test,

Committee on Methods for Toxicity Testing with Aquatic Organisms: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, EPA-660/3-75-009 (1975)

Müller, P. H.: Lexikon der Stochastik, Wissenschaftliche Buchgesellschaft, 2nd edition, (1975)

Elenk, B.-P.: Untersuchungen zur Ernährung von Daphnien; Dissertation, Heidelberg University (1990)

## 8 ORDER INFORMATION

Sponsor: Dr. Thömel                              Telephone: 49665  
Department code: CZN/EZ - E 100                      Date of order: 07 Dez 2000

## 9 SPECIFICATION OF THE TEST SUBSTANCE

Name of test substance : N-Methylpyrrolidon  
Chemical name : 1-Methyl-2-pyrrolidon  
Other names : NMP  
Batch number : continuous production from tank 53  
Date of production : 12 Oct 1993  
Product number : no details  
CAS number : 872-50-4  
Molecular formula : C<sub>5</sub>H<sub>9</sub>NO  
Molecular weight [g/mol] : 99.1  
Aggregate state : liquid  
Density [g/ml] : 1.028 (at 25°C)  
Water-solubility : miscible up to 10g/l  
Colour : colourless  
Purity of the test substance : 98,8 area-% [see 2]  
Impurities : 0,8% N-methyl-succinimide [see 2]  
Homogeneity : yes  
Instability against : oxygen, acid, alkali  
Storage conditions : under N<sub>2</sub>, ambient  
Expiry date: June 2007 [see 2]  
Further remarks : none

Origin of data of purity and homogeneity:

- 1) Observation of the Laboratory of experimental Toxicology and Ecology :
- 2) Report of the Analytical Laboratory ZAX No: 00L00368
- 3) Report of the sponsor No:
- 4) Report of the APD-Projekt:
- 5) Other Reports:

## 10 PRELIMINARY INVESTIGATIONS

In an acute preliminary test with daphnids the value for the EC<sub>50</sub> (48h) was > 100 mg/l.  
(Project-No.: 00/0969/50/v1, from 08 Jan 2001, non GLP)

## 11 TEST INFORMATION

### Water composition

A synthetic fresh water was used for culture and test purposes. This water was prepared on the basis of an ultrapure, demineralized water with a conductivity < 0.05 µS/cm. For the composition of this M4 medium see ISO 10706.

#### Properties of this medium:

|                         |   |                    |
|-------------------------|---|--------------------|
| Total hardness          | : | 2,20 - 3,20 mmol/l |
| Alkalinity up to pH 4.3 | : | 0,80 - 1,00 mmol/l |
| Molar ratio Ca:Mg       | : | about 4 : 1        |
| pH value                | : | 7,5 - 8,5          |
| Conductivity            | : | 550 - 650 µS/cm    |

After preparation the M4 medium is aerated for approximately 24 h until saturation with oxygen is reached. The measured minimum and maximum values of the physical and chemical parameters are given under clause 13 account of the test results.

### Feeding

During the test daphnids were fed live green algae (*Scenedesmus subspicatus* (= *Desmodesmus subspicatus*)), cultured in a synthetic medium daily. The algae were separated from culture medium by centrifugation, resuspended in daphnid's medium (M4) and daphnids were fed this concentrate. The algae were stored in a refrigerator (dark, about 4-8°C) for maximum 14 days. Through feeding the concentrate (maximum: 0.3 ml / 50 ml / day) the test solution was diluted in the worst case (Friday-Monday, 3 feedings) by 0.9 ml / 50 ml (= 1.8%).

### Feeding schedule:

| Day of Test | Amount of food per parent animal and day<br>[mg COD] * |
|-------------|--|
| 0-1         | 0,22   |
| 2-3         | 0,25   |
| 4-5         | 0,35   |
| 6-7         | 0,43   |
| 8 ff        | 0,75   |

\*COD: chemical oxygen demand

**Performance of the test**

|   |   |   |
|---|---|---|
| Experimental phase  | : | 14 Mar 2001 – 04 Apr 2001   |
| Begin of the study  | : | 08 Mar 2001   |
| Duration of the test  | : | 21 days   |
| Test temperature  | : | 18 - 22 °C (max. temperature difference 2 °C)   |
| Test vessel   | : | numbered glass beakers, nominal volume 100 ml, covered with numbered glass caps.  |
| Test volume   | : | 50 ml   |
| Loading   | : | 1 parent animal / 50 ml   |
| Number of animals/vessel  | : | 1   |
| Total number of animals/conc.                                       | : | 10  |
| Number of replicates/conc.  | : | 10  |
| Number of replicates/control  | : | 10  |
| Number of replicates for the measurement of pH and O <sub>2</sub>   | : | 1   |
| Number of parallels without animals                                 | : | 1 per test concentration and control for concentration control analysis.  |
| Age of the animals at the start of the test                         | : | 2 - 24 h (starting with the 3rd breed of parent animals)  |
| Age of stock animals  | : | 2 - 4 weeks   |
| Illumination  | : | artificial light, type warm white (e.g. OSRAM L58 W31)<br>day:night rhythm = 16 : 8 h   |
| Light intensity   | : | about 2 - 7 µE/(m <sup>2</sup> ·s) at a wave length of 400 - 700 nm   |
| Change of test solution   | : | each Monday, Wednesday and Friday   |
| Removal of young from test beakers and counting                     | : | daily   |
| Check of the study and recording (mortality, embryos, aborted eggs) | : | daily   |
| Feeding   | : | daily, according to feeding schedule (see page 7)   |
| Measurement of temperature  | : | Continuously during the whole test period in a separate vessel close to the test vessels.   |
| Measurement of pH and oxygen  | : | at the start of the test and at each change of test solution in the 48- or 72-hour-old solution in one parallel at each concentration |

#### Preparation of the stock solution and dilution

The test substance was stirred in M4 medium for about 30 minutes at  $20 \pm 2$  °C.

The nominal concentration of the stock solution was 100 mg/l.

By diluting this stock solution with M4 medium the following nominal concentrations (mg/l) were prepared:

|         |     |    |    |      |      |      |      |      |      |
|---------|-----|----|----|------|------|------|------|------|------|
| Control | 100 | 50 | 25 | 12,5 | 6,25 | 3,13 | 1,56 | 0,78 | 0,39 |
|---------|-----|----|----|------|------|------|------|------|------|

#### Statistics

For the statistical evaluation of the LOEC and NOEC Duncan's multiple range test was used.

#### Concentration control analyses

The concentration control analyses were performed with test solutions of the following nominal concentrations (mg/l) of the test substance:

|         |     |      |      |  |  |  |  |  |  |
|---------|-----|------|------|--|--|--|--|--|--|
| control | 100 | 12,5 | 1,56 |  |  |  |  |  |  |
|---------|-----|------|------|--|--|--|--|--|--|

Samples for analysis were taken in the 1st, the 2nd and the 3rd week of the test. For each concentration the freshly prepared test solution (unstocked) and the corresponding 48 h or 72 h old test solution (unstocked) were analyzed.

Detailed information on the methods of the concentration control analyses is given in the separate analytical test report (Annex).

#### 12 VALIDITY CRITERIA

- In the control the mortality of parent animals up to the end of the test was  $\leq 10\%$ .
- In the control no animals should be captured in the surface layer.
- In the control the first young animals should be observed till day 9.
- In the control the mean number of living young per parent animal that survived the test was  $\geq 60$ .
- In the control the coefficient of variation for the mean number of living young per parent animal that survived the test was  $\leq 25\%$ .
- The pH-value in the test solutions did not vary within more than one unit.
- The EC<sub>50</sub>(24h) of the control substance potassium dichromate was 0,92 mg/l  
(Date of the last control experiment: 14 Mar 2001, project number: 97/0242/50/55).

The test is valid.

### 13 ACCOUNT OF THE TEST RESULTS

Table 1

Summary of the NOEC and LOEC values for reproduction and LC<sub>0</sub> (mortality) after 21 d exposure.\*

NOEC = 12,5 mg/l

LOEC = 25 mg/l

LC<sub>0</sub> = 25 mg/l

\* see tables 4, 5, 6 and 7, for more detailed information

In the control the first young were observed at day 8. In the highest tested concentration, in which the daphnids produced young (100 mg/l), the first young were observed at day 8

Table 2

Minimum and maximum values of the chemical and physical characteristics of the test solutions during the test period.

| Parameter                | Minimum | Maximum |
|--------------------------|---------|---------|
| pH value **              | 7,4     | 8,2     |
| Oxygen content [mg/l] ** | 2,2     | 9,1     |
| Temperature [°C] *       | 20,3    | 21,0    |

\*\* measured in a separate vessel close to the test vessels

\* see tables 8 and 9 for more detailed information

Table 3

Analytically measured relative minimum and maximum concentrations of the test substance in the test solutions. The values are given in percent of the nominal concentrations\*.

| Time [h] | Stocked with daphnids | Minimum [%] | Maximum [%] |
|----------|-----------------------|-------------|-------------|
| 0        | No                    | 92,3        | 111         |
| 48/72    | no                    | 86,2**      | 126         |

\* The results are described in more detail in the separate analytical test report (Annex).

\*\* By preparing the reserve sample from concentration 12,5 mg/l (day 19) by mistake the sample was bottled wrong (recovery rate 0%). Because the recovery rate from the lowest (1,56 mg/l) and the highest concentration (100 mg/l) was within 100 ± 20 % the other concentrations including 12,5 mg/l have to be in the same range.

With an analytical recovery rate of > 80% no transformation of the nominal EC-values into the values of the concentration control analysis occurs.

**Table 4**

**Total number of living young per surviving parent animal after the 21 day exposure period.** Base for the data are the values standardized on the number of surviving parent animals of each of the ten parallels.

| concentration<br>[mg/l] | Par. 1 | Par. 2 | Par. 3 | Par. 4 | Par. 5 | Par. 6 | Par. 7 | Par. 8 | Par. 9 | Par. 10 | sum  | mean  | sd   | vc<br>[%] |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|------|-------|------|-----------|
| 0                       | 149    | 171    | 165    | 158    | 163    | 170    | 183    | 163    | 174    | 161     | 1857 | 165,7 | 8,9  | 5,4       |
| 0,39                    | 169    | 163    | 167    | 172    | 159    | 170    | 163    | 137    | 181    | 182     | 1663 | 166,3 | 12,0 | 7,2       |
| 0,78                    | 173    | 160    | 153    | 165    | 166    | 69     | 166    | 173    | 194    | 185     | 1804 | 160,4 | 32,5 | 23,2      |
| 1,56                    | 168    | 205    | 189    | 183    | T      | 170    | 160    | 173    | 139    | 164     | 1531 | 170,1 | 16,7 | 9,8       |
| 3,13                    | 171    | 157    | 151    | 154    | 173    | 163    | 146    | 153    | 176    | 148     | 1592 | 158,2 | 10,3 | 6,5       |
| 6,25                    | 174    | 169    | 147    | 144    | 142    | T      | 160    | 187    | 151    | 180     | 1454 | 161,6 | 15,7 | 8,7       |
| 12,5                    | 156    | 162    | 172    | 169    | 161    | T      | 182    | 171    | 161    | 188     | 1492 | 165,8 | 9,3  | 5,6       |
| 25                      | 121    | 110    | 126    | 146    | 134    | 104    | 141    | 136    | 169    | 131     | 1306 | 130,8 | 15,6 | 11,9      |
| 50                      | T      | 132    | 133    | T      | 99     | 100    | 107    | T      | 98     | T       | 669  | 111,5 | 15,1 | 13,6      |
| 100                     | T      | T      | 102    | T      | T      | T      | 98     | T      | 93     | T       | 293  | 97,7  | 3,7  | 3,8       |

**Table 5**

**Total number of dead young per surviving parent animal after the 21 day exposure period.** Base for the data are the values standardized on the number of surviving parent animals of each of the ten parallels.

| concentration<br>[mg/l] | Par. 1 | Par. 2 | Par. 3 | Par. 4 | Par. 5 | Par. 6 | Par. 7 | Par. 8 | Par. 9 | Par. 10 | sum  | mean | sd  | vc<br>[%] |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|------|------|-----|-----------|
| 0                       | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 0,39                    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 0,78                    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 1,56                    | 0      | 0      | 0      | 0      | T      | 0      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 3,13                    | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 6,25                    | 0      | 0      | 0      | 0      | 0      | T      | 0      | 0      | 2      | 0       | 2,0  | 0,2  | 0,8 | 282,8     |
| 12,5                    | 0      | 0      | 0      | 0      | 0      | T      | 0      | 0      | 0      | 0       | 0,0  | 0,0  | 0,0 | 0,0       |
| 25                      | 0      | 0      | 2      | 0      | 3      | 13     | 0      | 0      | 0      | 0       | 18,0 | 1,8  | 3,9 | 214,9     |
| 50                      | T      | 0      | 0      | T      | 4      | 1      | 11     | T      | 0      | T       | 16,0 | 2,7  | 4,0 | 149,5     |
| 100                     | T      | T      | 0      | T      | T      | T      | 0      | T      | 3      | T       | 3,0  | 1,0  | 1,4 | 141,4     |

T = Parent animal died during the test period

cv = coefficient of variance

sd = standard deviation

**Table 6**

**Total number of aborted subitane eggs per surviving parent animal after the 21 day exposure period.** Base for the data are the values standardized on the number of surviving parent animals of each of the four parallels.

| concentration<br>[mg/l] | Par. 1 | Par. 2 | Par. 3 | Par. 4 | Par. 5 | Par. 6 | Par. 7 | Par. 8 | Par. 9 | Par. 10 | sum | mean | sd  | vc<br>[%] |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|-----|------|-----|-----------|
| 0                       | 4      | 0      | 0      | 1      | 3      | 1      | 1      | 0      | 0      | 0       | 9   | 0,9  | 1,2 | 135,6     |
| 0,39                    | 0      | 0      | 2      | 1      | 1      | 1      | 0      | 3      | 1      | 2       | 11  | 1,1  | 0,9 | 85,8      |
| 0,78                    | 0      | 0      | 0      | 0      | 6      | 2      | 1      | 2      | 1      | 0       | 12  | 1,2  | 1,6 | 148,1     |
| 1,56                    | 1      | 0      | 5      | 0      | T      | 0      | 0      | 0      | 0      | 2       | 8   | 0,9  | 1,6 | 179,4     |
| 3,13                    | 1      | 0      | 0      | 2      | 3      | 0      | 0      | 0      | 1      | 0       | 7   | 0,7  | 1,0 | 143,6     |
| 6,25                    | 0      | 0      | 0      | 1      | 2      | T      | 2      | 1      | 0      | 0       | 6   | 0,7  | 0,8 | 122,5     |
| 12,5                    | 1      | 1      | 0      | 0      | 0      | T      | 1      | 0      | 1      | 1       | 5   | 0,6  | 0,5 | 89,4      |
| 25                      | 3      | 2      | 1      | 1      | 1      | 1      | 1      | 5      | 3      | 1       | 19  | 1,9  | 1,3 | 68,4      |
| 50                      | T      | 12     | 0      | T      | 3      | 1      | 8      | T      | 11     | T       | 38  | 5,8  | 4,7 | 81,3      |
| 100                     | T      | T      | 1      | T      | T      | T      | 0      | T      | 5      | T       | 6   | 2,0  | 2,2 | 108,0     |

T = Parent animal died during the test period

cv = coefficient of variance

sd = standard deviation

**Table 7**

Total number of surviving parent animals at various concentrations of the test substance and all days of the test.

| concentration<br>[mg/l] | day of the test |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|-------------------------|-----------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|                         | 0               | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 0                       | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 0,39                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 0,78                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1,56                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9  |
| 3,13                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6,25                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9  | 9  | 9  |
| 12,5                    | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9  |
| 25                      | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 50                      | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7  | 7  | 6  |
| 100                     | 10              | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7  | 4  | 4  | 3  |

\* died because of mechanical injury during change of test solution

**Table 8**

Measured values for the oxygen content [mg/l], measured in one sample per test concentration.

| concentration<br>[mg/l] | day of the test |     |     |     |     |     |     |     |     |     |
|-------------------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                         | 0               | 2   | 5   | 7   | 9   | 12  | 14  | 16  | 19  | 21  |
| 0                       | 8,9             | 8,9 | 8,5 | 8,3 | 8,6 | 8,4 | 8,7 | 8,3 | 8,5 | 8,2 |
| 0,39                    | 9,1             | 9,0 | 8,5 | 8,4 | 8,5 | 8,4 | 8,6 | 8,1 | 8,3 | 8,1 |
| 0,78                    | 9,0             | 9,0 | 8,6 | 8,3 | 8,4 | 8,3 | 8,7 | 8,0 | 8,1 | 8,2 |
| 1,56                    | 9,0             | 9,0 | 8,5 | 8,2 | 8,5 | 8,0 | 8,8 | 8,0 | 8,1 | 8,0 |
| 3,13                    | 9,0             | 8,9 | 8,7 | 8,4 | 8,1 | 8,2 | 8,7 | 8,1 | 8,5 | 8,1 |
| 6,25                    | 9,0             | 8,9 | 8,7 | 8,3 | 8,1 | 8,1 | 8,6 | 7,8 | 8,2 | 7,7 |
| 12,5                    | 9,0             | 8,9 | 8,4 | 8,1 | 8,1 | 8,0 | 8,5 | 7,4 | 8,3 | 7,0 |
| 25                      | 9,0             | 8,9 | 8,5 | 8,4 | 7,8 | 8,2 | 8,2 | 7,0 | 8,9 | 5,8 |
| 50                      | 9,0             | 8,8 | 8,8 | 8,3 | 7,6 | 8,1 | 7,5 | 5,8 | 6,1 | 7,3 |
| 100                     | 9,0             | 9,0 | 8,6 | 8,6 | 8,3 | 5,0 | 7,2 | 5,8 | 3,5 | 2,2 |

**Table 9**

pH-values, measured in one sample per test concentration.

| concentration<br>[mg/l] | day of the test |     |     |     |     |     |     |     |     |     |
|-------------------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                         | 0               | 2   | 5   | 7   | 9   | 12  | 14  | 16  | 19  | 21  |
| 0                       | 8,2             | 8,2 | 7,9 | 8,0 | 8,0 | 8,0 | 8,1 | 7,9 | 7,9 | 7,6 |
| 0,39                    | 8,2             | 8,2 | 7,9 | 8,0 | 8,0 | 8,0 | 8,1 | 7,9 | 7,8 | 7,6 |
| 0,78                    | 8,1             | 8,2 | 7,9 | 8,0 | 8,0 | 7,9 | 8,0 | 7,9 | 7,8 | 7,6 |
| 1,56                    | 8,1             | 8,2 | 7,9 | 7,9 | 8,0 | 7,9 | 8,0 | 7,8 | 7,8 | 7,6 |
| 3,13                    | 8,1             | 8,2 | 7,9 | 7,9 | 7,9 | 7,9 | 8,0 | 7,8 | 7,8 | 7,6 |
| 6,25                    | 8,1             | 8,1 | 7,9 | 7,9 | 7,9 | 7,9 | 8,0 | 7,8 | 7,8 | 7,6 |
| 12,5                    | 8,1             | 8,1 | 7,9 | 7,9 | 8,0 | 7,9 | 8,0 | 7,7 | 7,8 | 7,6 |
| 25                      | 8,1             | 8,1 | 7,9 | 7,9 | 7,4 | 7,9 | 8,0 | 7,7 | 7,9 | 7,5 |
| 50                      | 8,1             | 8,1 | 7,9 | 7,9 | 7,6 | 7,9 | 7,9 | 7,6 | 7,9 | 7,5 |
| 100                     | 8,1             | 8,1 | 7,9 | 7,9 | 7,7 | 7,8 | 7,9 | 7,6 | 7,5 | 7,4 |













Table 16

Table and summarized evaluation of the daily single values of the amount of young (total, alive and dead), of the aborted subitane eggs and the living parent animals.

Concentration of the test substance: 1,56 mg/l

| vessel No. | day | 0                  | 1                 | 2                  | 3                    | 4                  | 5                  | 6                 | 7                  | 8                    | 9                  | 10                 | 11                | 12                 | 13                   | 14                 | 15                 | 16                | 17                 | 18                   | 19                 | 20                 | 21                | sum                |                      |                    |
|------------|-----|--------------------|-------------------|--------------------|----------------------|--------------------|--------------------|-------------------|--------------------|----------------------|--------------------|--------------------|-------------------|--------------------|----------------------|--------------------|--------------------|-------------------|--------------------|----------------------|--------------------|--------------------|-------------------|--------------------|----------------------|--------------------|
|            |     | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> |
| 1          | 0   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 15                 | 0                    | 0                  | 31                 | 0                 | 0                  | 25                   | 0                  | 0                  | 47                | 0                  | 0                    | 50                 | 0                  | 168               |                    |                      |                    |
|            | 1   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            | 2   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 15                   | 0                  | 0                  | 31                | 0                  | 0                    | 26                 | 0                  | 0                 | 47                 | 0                    | 0                  | 50                 | 0                 | 168                |                      |                    |
|            | 2   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 1                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 1                    |                    |
|            | 3   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 205                  |                    |
|            | 3   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            | 4   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 14                   | 0                  | 21                 | 0                 | 8                  | 28                   | 3                  | 0                  | 48                | 0                  | 0                    | 48                 | 0                  | 0                 | 49                 | 0                    | 169                |
|            | 4   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            | 5   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 16                   | 0                  | 0                  | 31                | 0                  | 0                    | 37                 | 0                  | 0                 | 50                 | 0                    | 0                  | 49                 | 0                 | 183                |                      |                    |
|            | 5   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            | 6   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 14                   | 0                  | 0                  | 28                | 0                  | 0                    | 36                 | 0                  | 0                 | 48                 | 0                    | 0                  | 44                 | 0                 | 170                |                      |                    |
|            | 6   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            | 7   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 5                    | 0                  | 0                  | 28                | 0                  | 0                    | 38                 | 0                  | 0                 | 43                 | 0                    | 0                  | 46                 | 0                 | 160                |                      |                    |
|            | 7   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 5                    | 0                  | 0                  | 28                | 0                  | 0                    | 38                 | 0                  | 0                 | 43                 | 0                    | 0                  | 46                 | 0                 | 132                |                      |                    |
|            | 8   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 16                   | 0                  | 0                  | 27                | 0                  | 0                    | 39                 | 0                  | 0                 | 46                 | 0                    | 0                  | 45                 | 0                 | 173                |                      |                    |
|            | 8   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 16                   | 0                  | 0                  | 27                | 0                  | 0                    | 39                 | 0                  | 0                 | 46                 | 0                    | 0                  | 45                 | 0                 | 173                |                      |                    |
|            | 9   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 18                   | 0                  | 0                  | 33                | 0                  | 0                    | 33                 | 0                  | 0                 | 24                 | 0                    | 0                  | 30                 | 0                 | 139                |                      |                    |
|            | 9   | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 18                   | 0                  | 0                  | 33                | 0                  | 0                    | 33                 | 0                  | 0                 | 24                 | 0                    | 0                  | 30                 | 0                 | 139                |                      |                    |
|            | 10  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 17                   | 0                  | 0                  | 25                | 0                  | 0                    | 34                 | 0                  | 0                 | 47                 | 0                    | 0                  | 41                 | 0                 | 164                |                      |                    |
|            | 10  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 17                   | 0                  | 0                  | 25                | 0                  | 0                    | 34                 | 0                  | 0                 | 47                 | 0                    | 0                  | 41                 | 0                 | 164                |                      |                    |
| SUMMARY    |     | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> | Y <sub>total</sub> | Y <sub>dead</sub> | Y <sub>alive</sub> | S <sub>aborted</sub> | P <sub>alive</sub> |
|            |     | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 128                | 26                   | 21                 | 232                | 37                | 28                 | 284                  | 45                 | 46                 | 349               | 48                 | 37                   | 331                | 49                 | 1663              |                    |                      |                    |
|            |     | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            |     | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 128                | 26                   | 21                 | 232                | 37                | 28                 | 284                  | 45                 | 46                 | 349               | 48                 | 37                   | 331                | 49                 | 1663              |                    |                      |                    |
|            |     | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 5                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    | 5                  | 0                  | 0                 | 0                  | 0                    | 0                  | 0                  | 0                 | 0                  | 0                    |                    |
|            |     | 10                 | 10                | 10                 | 10                   | 10                 | 10                 | 10                | 10                 | 10                   | 10                 | 10                 | 10                | 10                 | 10                   | 10                 | 10                 | 10                | 10                 | 10                   | 10                 | 10                 | 9                 | 9                  | 9                    |                    |

P = Parent animal, S = Subitane egg, Y = Young

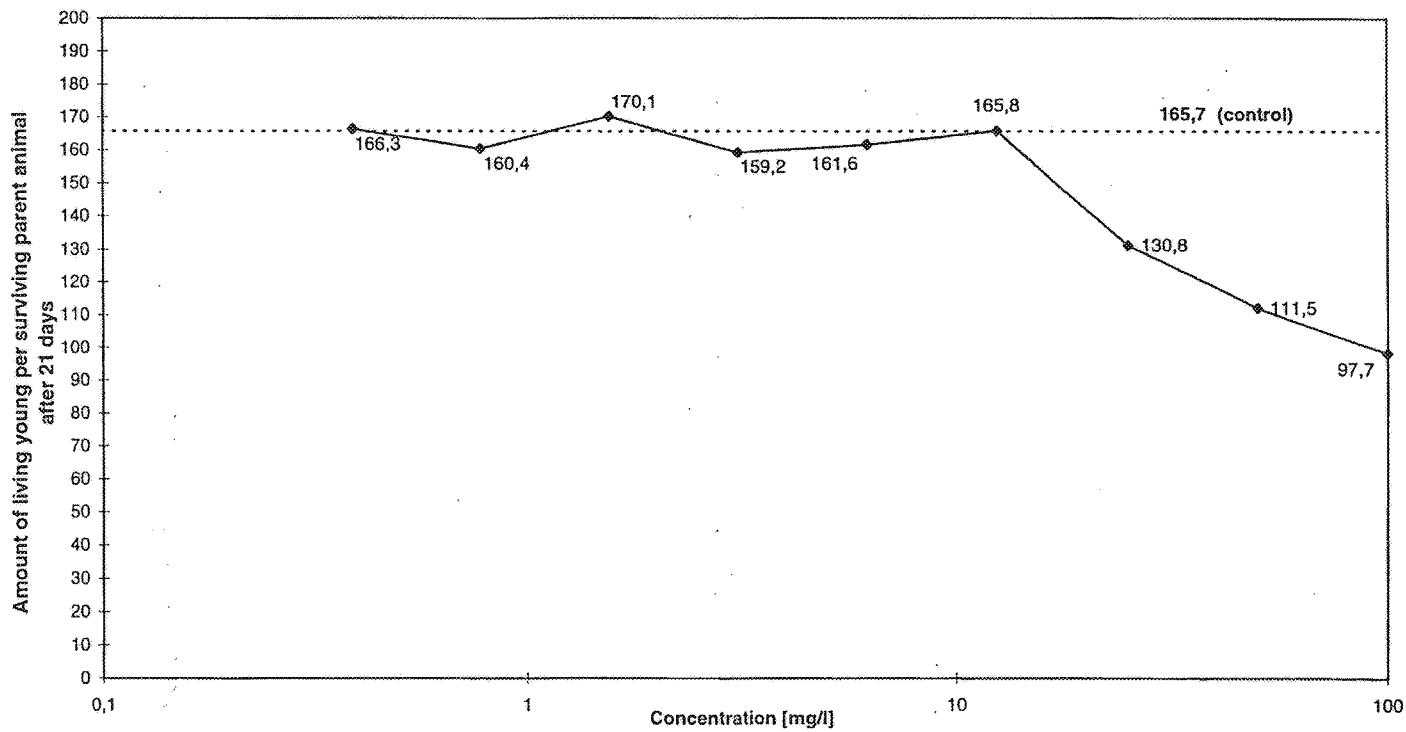
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**N-Methylpyrrolidon  
median reproduction rate**



**ANALYTICAL REPORT**

**No. 00/0969/51/1**

Test substance: N-Methylpyrrolidon  
Results: Values of days 0,2,7,9,16 and 19

## Concentration Control Analysis of N-Methylpyrrolidon in M4-Medium

### 1. SENDER

Sender: Dr. Jatzek  
Project No.: 00/0969/51/1  
Test substance: N-Methylpyrrolidon  
Batch No.: Continuous production from tank 53

### 2. SAMPLE DATA

#### 2.1. CONCENTRATION CONTROL ANALYSIS

Vehicle: M4-Medium  
Storage conditions of the samples until analysis: refrigerator

### 3. MATERIAL AND METHODS

#### 3.1. SAMPLE PREPARATION FOR ANALYSIS

Aliquots of the samples were directly used for HPLC-analysis.

#### 3.2. ANALYTICAL METHOD

HPLC with external calibration

Column: Nucleosil 100 5 CN 250x4mm  
Eluant: 10% Methanol  
          90% Natriumacetat pH 11  
Flow rate: 1.0 ml/min  
Detection: UV, 210 nm

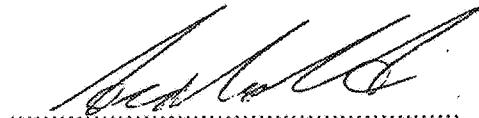






Analytical Results; Project No.: 00/0969/51/1

page 5 of 5

  
Dr. Leibold  
(Head of analytical laboratory)

  
Date  
(dd/mm/yyyy)

Note: The data given are part of Project 00/0969/51/1 which was performed in compliance with GLP and the results were audited by the QAU.